2/27/96

PHARMACOLOGIST'S REVIEW

PLA: 95-1167
SPONSOR: Boehringer Mannheim Pharmaceutical Corporation
PRODUCT: recombinant human plasminogen activator; reteplase; Rapilysin™; rPA
FORMULATION/CHEMISTRY: Produced in a bacterial (*E. coli*) expression system, consisting of the kringle 2 and protease domains of human tPA. A nonglycosylated protein with a MW of 39,571.5 daltons. The protein contains 355 of the 527 amino acids of native tPA. Formulated as a sterile lyophilized powder with L-arginine, phosphoric acid, and polysorbate 20. Each vial contains 10 U of reteplase.
PROPOSED INDICATION: For the management of AMI in adults for lysis of thrombi obstructing coronary arteries, the improvement of ventricular function following AMI, the reduction of the incidence of congestive heart failure, and the reduction of mortality associated with AMI.
ABBREVIATIONS: recombinant human plasminogen activator = reteplase = Rapilysin™; tissue plasminogen activator = tPA; acute myocardial infarction = AMI; thromboplastin time = TT

assigned 9/07/95; completed 01/23/96

CROSS-REFERENCES: ~~~~

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INTRODUCTION:

Reteplase, a "fibrin-selective" recombinant plasminogen activator derived from human tPA, catalyzes the cleavage of endogenous plasminogen to generate plasmin. This activation is stimulated in the presence of fibrin, resulting in degradation of the fibrin matrix of the thrombus. The sponsor claims that in clinical trials (RAPID 1) a decrease of 59% occurred in the fibrinogen levels at 4 hours postdose in the 10+10 U reteplase regimen compared to 42% for the 3-hour infusion of alteplase. In addition, the RAPID 2 angiographic study showed that the 10+10 U regimen provided more rapid and more complete thrombolysis compared to alteplase.

The proposed package insert submitted by the sponsor states that the recommended dosage for reteplase is as a 10+10 U double-bolus

initiation of treatment should begin as soon as possible after the onset of AMI symptoms.
Reteplase has a longer half-life than alteplase (effective half-life of 13-16 minutes), thus can be administered as two bolus injections, whereas administration of alteplase requires infusion.

PRECLINICAL PHARMACOLOGY STUDIES:

In vitro

List of Studies:

1. Differential Fibrinolytic Properties of the Recombinant Plasminogen Activator BM 06.022 in Human Plasma and Blood Clot Systems In Vitro; study #D13; performed at BM; and by Martin, U., et al., Blood Coagul. Fibrinolysis. 4:235-242, 1993; and by Martin, U., et al., Naunyn Schmiedeberg's Arch. Pharmacol. 347:(suppl):R94, 1993

2. Evaluation of the Synergistic Potential of BM 06.022 in Combination with Other Plasminogen Activators and of the Functional Binding of BM 06.022 to Fibrin in an In Vitro Clot Lysis System; study #D7; performed at BM

3. Biochemical Properties of the Kringle 2 and Protease Domains are Maintained in the Refolded tPA Deletion Variant BM 06.022; study #D15; performed at BM; and Kohnert, U., et al., Protein Eng. 5:93-100, 1992; study #D1015

4. A Variant of tPA Comprised of the Kringle 2 and the Protease Domain Shows a Significant Difference in the In Vitro Rate of Plasmin Formation as Compared to the Recombinant Human tPA from Transformed CHO Cells; study #D16; performed at BM; and Kohnert, U., et al., Fibrinolysis. 7:365-372, 1993; study #D1016; and Kohnert, U., et al., Fibrinolysis. 6(suppl 2):81, 1992.

5. Effects of the Novel Recombinant Plasminogen Activator BM 06.022 on Human Platelet Aggregation in Vitro; study #E16; performed at BM; and by Martin, U., et al., Fibrinolysis. 7:203-210, 1993 (study #E1016)

6. Comparisons of the Interaction of rPA and CHO-tPA with Endothelial and HepG2 Cells; study #E17; performed at BM

[Note that the reteplase potency units are based on an amidolytic assay. One megaunit (MU) from this assay equals a single reteplase unit (U) from the clot lysis assay, which is used by

Studies:

1. Differential Fibrinolytic Properties of the Recombinant Plasminogen Activator BM 06.022 in Human Plasma and Blood Clot Systems In Vitro; and by Martin, U., et al., Blood Coagul. Fibrinolysis. 4:235-242, 1993; and by Martin, U., et al., Naunyn Schmiedeberg's Arch. Pharmacol. 347:(suppl):R94, 1993
Clots were formed by adding $CaCl_2$ and human thrombin to human (fresh & aged) platelet poor plasma (PPP), platelet rich plasma (PRP), or fresh whole blood containing ^{125}I-fibrinogen. The clots were exposed to BM 06.022, alteplase (800,000 IU/mg), melanoma tPA (430,000 IU/mg), and urokinase (139,197 IU/mg) and immersed in plasma. Lysis was measured by ^{125}I release. Concentrations needed to achieve 50% clot lysis were 4.33, 10.36, 0.68, and 2.15 nM for BM 06.022, urokinase, alteplase, & mtPA, respectively. Maximal lysis rate was similar (84.1-87.6%) for all but urokinase (65.3%). The maximal lysis rate of BM 06.022 was lower than alteplase for fresh and aged PRP and whole blood clot lysis, but, although less potent (on a molar basis - 6.4-fold), displayed similar maximal efficacy (E_{max}) and degree of fibrin selectivity in fresh PPP-clot lysis.

Comments:
- [Per the sponsor] Based on the above study data, the less efficient lysing of PRP and whole blood clots by BM 06.022 compared to alteplase may favor a reduction in serious bleeding complications.

- [Per the sponsor] reteplase is more susceptible to inactivation in plasma by protease inhibitors (C1-inhibitor & α2-antiplasmin) than alteplase.

2. Evaluation of the Synergistic Potential of BM 06.022 in Combination with Other Plasminogen Activators and of the Functional Binding of BM 06.022 to Fibrin in an In Vitro Clot Lysis System
Fibrin clots were exposed to BM 06.022, urokinase, tPA, or E. coli-derived rtPA and lysis monitored (radiolabel counting). BM 06.022 had no potential for synergism with urokinase or E. coli rtPA (full-sequence) in vitro, and did not functionally bind to fibrin.

Comments:
- [Per the sponsor] The in vivo animal studies showed a higher thrombolytic potency than alteplase due to its PK profile - a component which is absent in vitro.

- [Per the sponsor] Reteplase is marginally bound to the fibrin of the clot, as transfer of the clot preincubated with the activator to fresh plasma (with no activator) resulted in the absence of further lysis. Thus there should be no sustained lysis in vivo after clearance from the circulation.

3. Biochemical Properties of the Kringle 2 and Protease Domains are Maintained in the Refolded tPA Deletion Variant BM 06.022; and Kohnert, U., et al., Protein Eng. 5:93-100, 1992
The reports contain information regarding the structure of kringle 2 and the protease compared to the structure of these domains in the intact tPA molecule. [Refer to the reports for further detail.]

4. A Variant of tPA Comprised of the Kringle 2 and the Protease Domain Shows a Significant Difference in the In Vitro Rate of Plasmin Formation as Compared to the Recombinant Human tPA from Transformed CHO Cells; and Kohnert, U., et al., Fibrinolysis. 7:365-372, 1993; and Kohnert, U., et al., Fibrinolysis. 6(suppl 2):81, 1992.
When using CNBr-fragments of fibrinogen as a stimulator, the activation of BM 06.022 was reduced compared to CHO-tPA. The rate of plasmin formation by these materials is dependent on exact concentration of the stimulator because the activity-stimulator curves of both enzymes are not parallel. However, in the presence of fibrin-monomer and plasmin-derived degradation products, the activity-stimulator curves were parallel, and similar concentrations of stimulator were needed to achieve both 50% and maximal stimulation for the products, although the in vitro rate of plasmin formation was ~3-fold lower for BM 06.022 compared to CHO-tPA.

5. Effects of the Novel Recombinant Plasminogen Activator BM 06.022 on Human Platelet Aggregation in Vitro; and by Martin, U., et al., Fibrinolysis. 7:203-210, 1993
Batch #821 699A; 556 kU/mg
Citrated human platelet-rich-plasma (PRP) was incubated with BM 06.022 at 400-3200 U/mL, alteplase at 800 kU/mg, or urokinase at 139,197 IU/mg, alone or in combination with saline, heparin, and/or acetylsalicylic acid. Platelet aggregation (ADP agonist) was reduced in a time- and dose-dependent manner with BM 06.022 alone. The platelet inhibitory effect was reduced/abolished with exposure to aprotonin or D-Phe-Pro-Arg-chloromethylketone (PPACK) [indicative of a plasmin-dependent mechanism]. Incubation of BM 06.022 + acetylsalicylic acid decreased platelet aggregation more

6. Comparisons of the Interaction of rPA and CHO-tPA with Endothelial and HepG2 Cells

Batch #G001, G008

The endothelial cells were obtained from human umbilical cords and platelets were fresh from healthy humans. ^{125}I-CHO-tPA, ^{125}I-BM 06.022, ^{125}I-urokinase were used.

Findings: At [redacted], the number of binding sites ([redacted] per endothelial or HepG2 cell was 10-fold lower for ^{125}I-BM 06.022 compared to tPA.

At 37°C, ^{125}I-BM 06.022 has 5-fold less low affinity binding sites and 2-fold less high affinity binding sites compared to tPA.

Degradation of ^{125}I-BM 06.022 occurs mainly in the lysosomes of HepG2 cells and ~40% in the lysosomes of endothelial cells.

BM 06.022 and tPA share some of the same binding sites.

LDL receptor related protein is involved in internalization of BM 06.022 in endothelial and HepG2 cells and in binding and internalization in mesothelial cells.

Plasminogen activator inhibitor is involved in the specific interaction of BM 06.022 with the cells.

<u>In vivo</u>

List of Studies:

1. Thrombolytic Action of BM 06.022 in a Plasma Clot Assay, a Rat Pulmonary Embolism Model, and a Rabbit Model of Jugular Vein Thrombosis; study #D10; performed at BM

2. Thrombolytic Potency of BM 06.022 After Systemic IV Infusion in a Rabbit Model of Jugular Vein Thrombosis; study #D1; performed at BM

3. Thrombolytic Effects of BM 06.022 One Hour After a Single IV Bolus Injection in a Rabbit Model of Jugular Vein Thrombosis; study #D4; performed at BM

4. Thrombolytic Potency of BM 06.022 Two Hours After a Single IV Bolus Injection in a Rabbit Model of Jugular Vein Thrombosis; study #D5; performed at BM

5. Thrombolysis with an E. coli-Produced Recombinant Plasminogen Activator (BM 06.022) in the Rabbit Model of Jugular Vein

6. Dose-Response Effectiveness of BM 06.022 After Single IV Bolus Injection in a Canine Stenotic Model of Coronary Artery Thrombosis; study #D2; performed at BM

7. Coronary Thrombolytic Properties of a Novel Recombinant Plasminogen Activator (BM 06.022) in a Canine Model; study #D1002; Martin, U., et al., J. Cardiovasc. Pharmacol. 18:111-119, 1991

8. Coronary Thrombolysis in Dogs Following Bolus Injection of Recombinant Plasminogen Activator BM 06.022; study #D1002; Martin, U., et al., J. Cardiovasc. Pharmacol. 18:111-119, 1991

9. PD Characterization of BM 06.022 in a Canine Stenotic Model of Coronary Artery Thrombosis; study #D6; performed at BM

10. Comparison of Thrombolytic Effectiveness of BM 06.022 After IV Injection Versus Infusion in a Canine Stenotic Model of Coronary Artery Thrombosis; study #D3; performed at BM

11. Thrombolytic Potency of an E. coli-Produced Novel Variant of rt-PA in Dogs; study #D1003; Martin, U., et al., Fibrinolysis. 4(suppl 3):9, 1990

12. Evaluation of Thrombolytic and Systemic Effects of the Novel Recombinant Plasminogen Activator BM 06.022 Compared with Alteplase, Anistreplase, Streptokinase, and Urokinase in a Canine Model of Coronary Artery Thrombosis; study #D9; performed at BM and by Martin, U., et al., J. Am. Coll. Cardiol. 19:433-440, 1992

13. Comparative Evaluation of Thrombolytic Properties of a Novel Recombinant Plasminogen Activator in Dogs; study #D1009; Martin, U., et al., J. Am. Coll. Cardiol. 17:26A, 1991

14. Coronary Thrombolytic Properties of the Recombinant Plasminogen Activator BM 06.022 in Dogs; study #D1109; Martin, U., et al., Ann. Hematol. 62:A75, 1991

15. Double Bolus Administration of BM 06.022 Improves Coronary Blood Flow After Reperfusion in a Canine Model of Coronary Artery Thrombosis; study #D8; performed at BM; and by Martin, U., et al., Blood Coagul. Fibrinolysis. 3:139-147, 1992 (study #D1008)

16. Sulotroban and Hirudin Improve Coronary Blood Flow After Reperfusion Induced by BM 06.022 in a Canine Model of Coronary Artery Thrombosis; study #D11; performed at BM; and by Martin, U., et la., Int. J. Hematol. 56:143-153, 1992

18. Effects of Sulotroban on Cyclic Flow Reduction After Reperfusion by a Recombinant Plasminogen Activator (BM 06.022) in Dogs; study #R24; Martin, U., et al., Naunyn Schmiedeberg's Arch. Pharmacol. 342:(suppl):88, 1990

19. Influence of Heparin and Systemic Lysis on Coronary Blood Flow After Reperfusion Induced by the Novel Recombinant Plasminogen Activator BM 06.022 in a Canine Model of Coronary Thrombosis; study #D14; performed at BM; and by Martin, U., et al., Naunyn Schmiedeberg's Arch. Pharmacol. 345:(suppl 2):R62, 1992

20. Rapid Reversal of Canine Thromboembolic Pulmonary Hypertension by Bolus Injection of the Novel Recombinant Plasminogen Activator BM 06.022; study #D12; performed at BM

21. Rapid Reversal of Canine Thromboembolic Pulmonary Hypertension by Bolus Injection of the Novel Recombinant Plasminogen Activator BM 06.022; study #D1012; Martin, U., et al., J. Cardiovasc. Pharmacol. 21:455-461, 1993; and by Martin, U., et al., Fibrinolysis. 6(suppl 2):139, 1992

22. General Pharmacology Study of BM 06.022; study #E14; performed at BM

23. Evaluation of BM 06.022 in the Irwin Behavioral Test in Mice (IV Administration); study #E9; performed at BM

24. Effect of BM 06.022 on Locomotoric Activity in the Mouse; study #E10; performed at BM

25. Effect of BM 06.022 on Pentetrazole-Induced Convulsions in Mice (IV Administration); study #E12; performed at BM

26. Effects of BM 06.022 on the Hemodynamic and Hemostasis System, on Blood Count, and on the Respiratory Function in Conscious Rabbits; study #E8; performed at BM

27. Hemodynamic Effects of BM 06.022 in Conscious Dogs; study #E3; performed at BM

28. Hemodynamic Effects of BM 06.022 in a Canine Stenotic Model of Coronary Thrombosis; study #E4; performed at BM

29. Hemodynamic Effects of High Doses of BM 06.022 in Conscious Dogs; study #E7; performed at BM

30. Antigenicity Study of BM 06.022 in Guinea Pigs; study #E13;

31. Comparative Evaluation of the Antigenicity of BM 06.022 in Dogs; study #E11; performed at BM

32. Fibrin-Selectivity of BM 06.022 in Rabbits After Single IV Bolus Injection; study #E5; performed at BM

33. Effects of the Novel Recombinant Plasminogen Activator BM 06.022 on Platelets and Bleeding Time in Rabbits; study #E15; performed at BM; and by Martin, U., et al., Platelets. 3:247-253, 1992. (study #E1015)

34. Effects of BM 06.022 on Simplate Bleeding Time in Anesthetized Dogs; study #E2; performed at BM

35. Effects of BM 06.022 on Hemostasis Parameters After IV Administration to Anesthetized Dogs; study #E6; performed at BM

36. Effects of BM 06.022 on the Hemostatic System After Single IV Bolus Injection into Non-Human Primates; study #E1; performed at BM

Studies:

1. Thrombolytic Action of BM 06.022 in a Plasma Clot Assay, a Rat Pulmonary Embolism Model, and a Rabbit Model of Jugular Vein Thrombosis
In the plasma clot lysis assay, BM 06.022 showed dose-dependent fibrinolysis and dose-dependent consumption of α2-plasmin inhibitor that was proportional to clot lysis (the binding of the material to fibrin is weaker compared to natural tPA). Dose-dependent thrombolysis was induced in both animal models.

2. Thrombolytic Potency of BM 06.022 After Systemic IV Infusion in a Rabbit Model of Jugular Vein Thrombosis
A 4-hour infusion of BM 06.022 and alteplase resulted in a dose-dependent increase in the thrombolysis rate. The ED_{50} was 94 kU/kg and 436 kU/kg for BM 06.022 and alteplase, respectively.

3. Thrombolytic Effects of BM 06.022 One Hour After a Single IV Bolus Injection in a Rabbit Model of Jugular Vein Thrombosis
NZW rabbits were IV injected with BM 06.022 at 50, 100, 200, & 400 kU/kg (1 min); alteplase at 200, 400, 800, & 1600 kU/kg (1 min); or anistreplase at 70, 280, & 560 μg/kg (1 min). A dose-dependent increase in the thrombolysis rate occurred. The ED_{50} was 183 kU/kg, 844 kU/kg, and 586 μg/kg for BM 06.022, alteplase,

4. Thrombolytic Potency of BM 06.022 Two Hours After a Single IV Bolus Injection in a Rabbit Model of Jugular Vein Thrombosis; and by Martin, U., et al., Thromb. Haemost. 65:560-564, 1991.
NZW rabbits were IV injected with BM 06.022 at 50, 100, 200, & 400 kU/kg (15 sec) and alteplase at 400, 800, & 1600 kU/kg (15 sec). A dose-dependent increase in the thrombolysis rate occurred. The ED_{50} was 163 kU/kg and 871 kU/kg for BM 06.022 and alteplase, respectively. The extent of thrombolysis correlated with $AUC_{0-2\ hr}$ for both materials; however, alteplase needed higher AUC values to obtain the same degree of thrombolysis as BM 06.022. At 50% thrombolysis, the amount of residual fibrinogen was 74.2% vs. 76.5% for BM 06.022 and alteplase, respectively.

5. Kinetic Studies of BM 06.022, a Plasminogen Activator Mutein Produced in E. coli; Kresse, GB., et al., J. Cell Biochem. suppl. 18D:157, 1994
[note that the one-page abstract was of poor copy quality]
BM 06.022 and alteplase have identical plasminogen activation activity in the absence of any stimulator. In the presence of fragments of fibrinogen, the in vitro catalytic efficiency of BM 06.022 is lower by a factor of 4, so that higher stimulator concentrations are needed for maximal activity compared to tPA. However, equal doses of both materials in rabbits results in similar decreases in fibrinogen and α_1-antiplasmin plasma concentrations, indicating sufficient fibrin specificity in vivo.

6. Dose-Response Effectiveness of BM 06.022 After Single IV Bolus Injection in a Canine Stenotic Model of Coronary Artery Thrombosis
The left circumflex coronary artery (LCX) of beagle dogs was occluded and thrombi were produced by applying a 150 μA anodal current to a wire placed within the lumen of the LCX just proximal to the screw occluder clamp. The heparinized dogs were IV injected with BM 06.022 at 50, 100, 140, & 200 kU/kg (1 min) or alteplase at 200, 800, 1130, & 1600 kU/kg (1 min). A dose-dependent increase in the thrombolysis rate occurred. The ED_{50} was 83 kU/kg and 961 kU/kg for BM 06.022 and alteplase, respectively.

7. Coronary Thrombolytic Properties of a Novel Recombinant Plasminogen Activator (BM 06.022) in a Canine Model; Martin, U., et al., J. Cardiovasc. Pharmacol. 18:111-119, 1991
Results from study list #6 published.

8. Coronary Thrombolysis in Dogs Following Bolus Injection of Recombinant Plasminogen Activator BM 06.022; Martin, U., et al., J. Cardiovasc. Pharmacol. 18:111-119, 1991
Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with BM 06.022 at 50, 100, 140, & 200 kU/kg. Dose-dependent reperfusion was noted in 2/6, 3/6, 4/6, or 6/6 dogs, respectively, compared to vehicle (0/6 dogs). The residual fibrinogen concentration decreased from 95% to 81%.

9. PD Characterization of BM 06.022 in a Canine Stenotic Model of Coronary Artery Thrombosis
Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with BM 06.022 at 140 kU/kg (1 min) or with alteplase at 800 kU/kg (1 min). Reperfusion was noted in 4/6 BM 06.022 dogs within 18 ± 6 min after injection and in an additional dog at 140 min. A total of 2/6 alteplase dogs showed reperfusion within 35 ±17 min of injection, plus an additional dog at 156 min. No vehicle dogs showed reperfusion. The $AUC_{0\text{-}2\ hr}$ of BM 06.022-treated animals was 397 ±49 IU.mL/hr and was 630 ±55 IU.mL/hr for alteplase, showing that initially high plasma concentrations after injection did not contribute to high lysis rates. PLT count, PLT aggregation, and bleeding time were not affected by BM 06.022.

10. Comparison of Thrombolytic Effectiveness of BM 06.022 After IV Injection Versus Infusion in a Canine Stenotic Model of Coronary Artery Thrombosis
Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with BM 06.022 at 50 or 140 kU/kg (1-min bolus or 90-min infusion) or with alteplase at 800 kU/kg (1-min bolus or 90-min infusion). Reperfusion was noted in 33% and 66% of the dogs dosed with alteplase via bolus and infusion, respectively. Reperfusion was noted in 0/4 dogs infused with 50 kU/kg of BM 06.022 compared to bolus injection (2/6 dogs). At 140 kU/kg of BM 06.022 - both injected and infused - 4/6 dogs were affected. However, at 140 kU/kg, the mean time to reperfusion was 59 min (infused dose) vs. 12 min (injected dose). No vehicle dogs showed reperfusion. The $AUC_{0\text{-}2\ hr}$ of 140 kU/kg BM 06.022-bolus injected dogs was 554 ±62 IU.mL/hr and was 490 ±85 IU.mL/hr for alteplase infusion.

Comment:
- The IV bolus injection of 140 kU/kg BM 06.022 was equally effective to the 90-minute infusion of 800 kU/kg alteplase (1 mg/kg).

11. Thrombolytic Potency of an E. coli-Produced Novel Variant of rt-PA in Dogs; Martin, U., et al., Fibrinolysis. 4(suppl 3):9, 1990
Results from study list #10 published.

12. Evaluation of Thrombolytic and Systemic Effects of the Novel Recombinant Plasminogen Activator BM 06.022 Compared with Alteplase, Anistreplase, Streptokinase, and Urokinase in a Canine Model of Coronary Artery Thrombosis; Martin, U., et al., J. Am. Coll. Cardiol. 19:433-440, 1992
Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with BM 06.022 at 140 kU/kg (1 min); alteplase at 800 kU/kg (1 min); anistreplase at 0.4 U/kg (1 min); streptokinase at 21,000 IU/kg (60 min); or urokinase at 20,000 IU/kg (90 min). Reperfusion was noted in 4/6 BM 06.022 dogs (18.3 ± 6 min); 2/6 alteplase dogs (19 & 52 min); 5/6 anistreplase dogs (57.8 ±12.1 min); 3/6 streptokinase dogs (67 ±12 min); and in 3/6 urokinase dogs (84.3 ±17.1 min). Reocclusion occurred in most of the reperfused dogs, but was prevented longer in the anistreplase dogs compared to BM 06.022 dogs. Residual fibrinogen was lower in anistreplase & urokinase dogs compared to BM 06.022, which was similar to alteplase & streptokinase dogs. Bleeding times were similar in BM 06.022 & alteplase dogs, but was longer in the other groups.

Comments:
- The human therapeutic dose of anistreplase is 30 U. The doses of the various thrombolytics used were based on results of clinical trials.

- IV bolus injection of BM 06.022 induced reperfusion at a high rate, but the problem of reocclusion (seen with other thrombolytics) was not resolved.

13. Comparative Evaluation of Thrombolytic Properties of a Novel Recombinant Plasminogen Activator in Dogs; Martin, U., et al., J. Am. Coll. Cardiol. 17:26A, 1991 17:26A, 1991
Results from study list #12 published.

14. Coronary Thrombolytic Properties of the Recombinant Plasminogen Activator BM 06.022 in Dogs; Martin, U., et al., Ann. Hematol. 62:A75, 1991
Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with BM 06.022 at 140 kU/kg;

alteplase at 800 kU/kg; or anistreplase at 0.4 mg/kg. Reperfusion was noted in 4/6 BM 06.022 dogs (18 ± 6 min); 2/6 alteplase dogs (35 ±17 min); and in 5/6 anistreplase dogs (58 ±12 min). Residual fibrinogen was lower in anistreplase dogs compared to BM 06.022, which was similar to alteplase dogs. Bleeding times were similar in BM 06.022 & alteplase dogs, but was longer in anistreplase dogs. The half-life activity of BM 06.022 was 13 min compared to alteplase at 1.7 min.

15. Double Bolus Administration of BM 06.022 Improves Coronary Blood Flow After Reperfusion in a Canine Model of Coronary Artery Thrombosis; and by Martin, U., et al., Blood Coagul. Fibrinolysis. 3:139-147, 1992

Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with BM 06.022 at 140 or 280 kU/kg (1 min); or with BM 06.022 at 140 **and** 140 kU/kg or 140 **and** 50 kU/kg (44 min apart per injection). Double bolus injection achieved a longer patency time (~123 min) compared to single injection (48 min at 140 kU/kg & 80 min at 280 kU/kg). Maximum coronary blood flow was ~20 min after double bolus injection vs. ~12 min for single injection. Residual fibrinogen was higher (~96 %) after double injection vs. single injection (~74%) at 140 kU/kg.

16. Sulotroban and Hirudin Improve Coronary Blood Flow After Reperfusion Induced by BM 06.022 in a Canine Model of Coronary Artery Thrombosis; and by Martin, U., et al., Int. J. Hematol. 56:143-153, 1992

Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with acetylsalicylic acid + BM 06.022 (140 kU/kg); sulotroban (10 mg/kg/hr) + BM 06.022 (140 kU/kg); or hirudin (1 mg/kg/hr) + BM 06.022 (140 kU/kg). Sulotroban and hirudin concomitant with BM 06.022 prolonged patency time (147 min in 4/6 dogs and 129 min in 7/8 dogs, respectively) compared to saline + BM 06.022 (48 min in 4/6 dogs) and acetylsalicylic acid + BM 06.022. Maximum coronary blood flow paralleled patency time findings. However, APTT was prolonged with hirudin + BM 06.022 compared to other groups. Bleeding times were longer for BM 06.022 + the 3 agents (from 3.8-15.5 min) compared to saline + BM 06.022 (2.5 min).

17. Hirudin Improves Coronary Blood Flow After Thrombosis by the tPA Variant BM 06.022; Martin, U., et al., Arterioscler. Thromb. 11:1575A, 1991

Results from study list #16 published.

18. Effects of Sulotroban on Cyclic Flow Reduction After Reperfusion by a Recombinant Plasminogen Activator (BM 06.022) in Dogs; Martin, U., et al., Naunyn Schmiedeberg's Arch. Pharmacol. 342:(suppl):88, 1990

Heparinized beagle dog models of electrically induced coronary thrombosis were IV injected with acetylsalicylic acid + BM 06.022 (140 kU/kg); sulotroban (10 mg/kg/hr) + BM 06.022 (140 kU/kg); saline + alteplase ((800 kU/kg); or saline + BM 060022. Sulotroban + BM 06.022 prolonged patency time (119 min) compared to all other combinations. However, the bleeding time was longer for sulotroban + BM 06.022 (8.3 min) compared to the other groups (from 2.3 - 3.8 min). Collagen-induced platelet aggregation was 5% for sulotroban + BM 06.022 compared to acetylsalicylic acid + BM 06.022 (13%) and saline + BM 06.022 (79%).

19. Influence of Heparin and Systemic Lysis on Coronary Blood Flow After Reperfusion Induced by the Novel Recombinant Plasminogen Activator BM 06.022 in a Canine Model of Coronary Thrombosis; and by Martin, U., et al., Naunyn Schmiedeberg's Arch. Pharmacol. 345:(suppl 2):R62, 1992

Beagle dog models of electrically induced coronary thrombosis were treated as follows: 1) heparin infusion (100 IU/kg/hr) + bolus BM 06.022 (200 kU/kg); 2) heparin bolus (200 IU/kg) + bolus BM 06.022 (200 kU/kg); 3) saline bolus + BM 06.022 (1000 kU/kg); or 4) saline infusion + BM 06.022 (200 kU/kg). Reperfusion was achieved in 100% of the group 1 & 2 dogs and in 70-86% of the group 3 & 4 dogs. Reocclusion occurred in all groups and was highest in group 4. Heparin infusion prolonged patency time compared to saline infusion (204 min vs. 35 min). Residual fibrinogen was similar for heparin infusion, heparin bolus, and saline infusion (91-100%), with a decrease for saline bolus (3%).

Comments:

- [Per the sponsor] A bolus dose of 200 kU/kg of BM 06.022 corresponds to the effective clinical dose of 15 MU in myocardial infarction patients. In the dogs, 200 kU/kg induced marginally reduced (~10%) fibrinogen, but in humans a 15 MU dose reduced plasma fibrinogen by ~50%, accompanied by increased numbers of fibrinogen degradation products (FDP), which can have an anticoagulant activity.

- The sponsor states that other thrombolytics use conjunctive treatment such as aspirin and IV heparin in order to reduce reocclusion. Heparin can also increase the risk for major non-cerebral bleeding and cerebral hemorrhage. The prolonged half-life of BM 06.022 (19 min) in humans allows for bolus injection.

Previous studies have shown that BM 06.022 induces a rapid reperfusion in coronary thrombosis, but due to the early reperfusion, reocclusions occur by 90 minutes in some cases. In this study, the sponsor was attempting to determine how best the restored blood flow could be maintained. The sponsor suggests that a single bolus injection of heparin as an adjunct to BM 06.022 may be beneficial in overcoming re-thrombosis during the [apparent] crucial initial 90 minute vulnerable phase. In addition, further use of heparin should be determined based on individual residual fibrinogen levels and APTT.

20. Rapid Reversal of Canine Thromboembolic Pulmonary Hypertension by Bolus Injection of the Novel Plasminogen Activator BM 06.022

Heparinized beagle dogs were IV injected with autologous thrombi into the femoral vein to induce embolic pulmonary hypertension. The dogs were single IV injected with BM 06.022 at 140 or 200 kU/kg (1 min); double bolus injection of 140 and 60 kU/kg (1 min each, 30 min apart); alteplase (1 mg/kg for 15 min, followed by 1.33 mg/kg for 2 hr); urokinase (13,333 U/kg for 10 min, followed by 26,667 U/kg for 110 min); and anistreplase (0.4 U/kg for 1 min). At 15 minutes postinjection, no differences in pulmonary artery pressure (PAP) were noted. At 30 minutes, PAP was significantly greater for 200 kU/kg BM 06.022 compared to other treatments, but by 3 hours, effects on PAP were equivalent for all groups.

A bolus injection of BM 06.022, resulted in C_{max} levels higher than alteplase (4498 ±716 vs. 519 ±119 IU/mL), thus resulting in more rapid lysis of the emboli. Reptilase times were shorter for BM 06.022 dogs compared to other treatments. There was a trend toward decreased PAP for the higher BM 06.022 dose - noted more at 3 hours than 30 minutes. [Per the sponsor] The bolus may be adequate for a rapid effect and a high dose or double dose may be desired for prolonged action.

Comment:

- Reptilase time is prolonged by fibrin and FDP, but not heparin.

21. Rapid Reversal of Canine Thromboembolic Pulmonary Hypertension by Bolus Injection of the Novel Recombinant Plasminogen Activator BM 06.022; and Martin, U., et al., J. Cardiovasc. Pharmacol. 21:455-461, 1993; and by Martin, U., et al., Fibrinolysis. 6(suppl 2):139, 1992

Results from study list #20 published.

22. General Pharmacology Study of BM 06.022; study #E14; performed at BM

Effects on CNS - Batch #821 699A (556 U/mg)
ddY-strain ♂ mice (10/grp) were single IV injected with 0.25, 0.75, or 2.5 mg/kg BM 06.022. No adverse effects were noted in gross behavior, spontaneous locomotor activity, sleeping time (induced by thiopental), maximal electric-shock convulsion response, drug-induced convulsions, and analgesic activity (by tail pinch). Acetic acid-induced writhing counts decreased to ~50% in the 0.75 & 2.5 mg/kg grps.
NZW ♂ rabbits (10/grp) singly IV injected with 0.25, 0.75, or 2.5 mg/kg BM 06.022 displayed no abnormal body temperatures.

Effects on Cardiovascular System - Batch #821 699A (556 U/mg)
Male beagle dogs (3/grp) were single IV injected with 0.25, 0.75, or 2.5 mg/kg BM 06.022. No changes in ECG, heart rate, or respiratory rate were noted. Transient drops in blood pressure (compared to baseline) occurred in some treated dogs - marked in the 2.5 mg/kg dogs - and the femoral artery blood flow transiently increased, followed by decrease. [No concurrent control was run.]
The sponsor notes that blood pressure should be closely monitored when increasing the clinical dose higher than the 0.35 mg/kg level.

Effect on ANS & Smooth Muscle - Batch #821 699A (556 U/mg)
ddY-strain ♂ mice (10/grp) were single IV injected with 0.25, 0.75, or 2.5 mg/kg BM 06.022, followed by 10 mL/kg of charcoal suspension. The mice were killed 30 minutes later and the digestive tracts removed. BM 06.022 was found to significantly suppress charcoal passage rates more than 10% compared to saline controls. [No concurrent vehicle controls were run.]

The ileum of Hartley ♂ guinea pigs (5/grp) was suspended (1 gm tension) in bicarbonate buffer and exposed to 2 x 10^{-6}, x 10^{-7}, or x 10^{-8} molar BM 06.022, followed by measurement of tension change. No test material related effects occurred.

The ileum of Hartley ♂ guinea pigs (5/grp) was suspended (1 gm tension) in bicarbonate buffer and exposed to 2 x 10^{-6}, x 10^{-7}, or x 10^{-8} molar BM 06.022, followed (1 minute later) by addition of agonists - 10^{-7} M acetylcholine chloride; 10^{-6} M histamine diphosphate; & 10^{-3} M $BaCl_2$. No test material related tension changes occurred.

Effect on Renal Function - Batch #821 699A (556 U/mg)
SD-strain SPF ♂ rats (6/grp) were single IV injected with 0.25, 0.75, or 2.5 mg/kg BM 06.022, followed by urine collection. No

Comment:
• Sleeping time - Thiopental was IV injected 5 minutes post-BM 06.022 dosing. The time required to restore normal posture from a supine position was determined.

Anti-convulsion - electric shock - 5 minutes post-treatment, the cornea was electrically connected to an electric shock apparatus, and the convulsions, comas, deaths recorded.

Anti-convulsion - drug-induced - 5 minutes post-treatment, SC injection of 85 mg/kg pentylenetetrazol, and the convulsions, comas, deaths recorded.

Analgesic activity - IP injection of 10 mL/kg of 0.6% acetic acid 5 minutes post-treatment, and the writhing counts measured.

Analgesic activity - SC injection of morphine, followed by test material. The tail is pinched by forceps.

23. Evaluation of BM 06.022 in the Irwin Behavioral Test in Mice (IV Administration)
Batch #821 563A
SPF/NMRI ♀ mice (6/grp) were single IV injected with 1.4 or 4.2 MU/ kg BM 06.022. [**Note** that 1.4 MU/kg is 10-fold higher required for a thrombolytic effect.] Reference mice were injected with methamphetamine (stimulant) or diazepam (sedative). No adverse effects were noted at 1.4 MU/kg alone. Mice treated at 4.2 MU/kg alone reacted similar to those treated with methamphetamine - increased alertness, vocalization, restlessness, reduced pinna reflex. The mice treated with diazepam showed staggering gait, reduced limb tone and reflex sensitivity.

24. Effect of BM 06.022 on Locomotoric Activity in the Mouse
Batch #821 563A
SPF/NMRI ♀ mice (6/grp) were single IV injected with 0.14, 0.42, 1.4, or 4.2 MU/kg BM 06.022 and locomotoric activity was measured.
Reference mice were injected with methamphetamine or diazepam. No effect on activity was noted at 0.14 MU/kg, while a slight stimulation of locomotoric activity was noted at the other BM 06.022 dose levels. Reference mice displayed expected results -increased/decreased activity with methamphetamine/diazepam.

25. Effect of BM 06.022 on Pentetrazole-Induced Convulsions in Mice (IV Administration)
Batch #821 563A & 564A
SPF/NMRI ♀ mice were single IV injected with 4.2 MU/kg BM 06.022, followed (15 minutes later) by IV injection of doxapram + pentetrazole. BM 06.022 did not influence pentetrazole-induced convulsions, as the intensity of the convulsions were similar to placebo controls. Doxapram controls increased convulsion intensity.

26. Effects of BM 06.022 on the Hemodynamic and Hemostasis System, on Blood Count, and on the Respiratory Function in Conscious Rabbits
Batch #821 403A (lyophilized); 578 kU/mg
NZW ♂ rabbits were single IV injected at 0.14, 0.42, 1.4, or 4.2 MU/kg BM 06.022 (1 min). No effects on heart rate, arterial blood pressure, arterial blood gases, acid-base balance, or CBCs were noted. Hemostasis proteins [fibrinogen, plasminogen, α2-antiplasmin - were dose-dependently reduced, and PTTs were prolonged.

Comment:
- [Per the sponsor] The dose of 4.2 MU/kg is 30-fold the therapeutic dose.

27. Hemodynamic Effects of BM 06.022 in Conscious Dogs
Batch #9/89
Adult mongrel dogs with chronically implanted catheters were single IV injected at 200,000 IU/kg BM 06.022 (1 min) or 200,000 IU/kg alteplase. No changes in heart rate, arterial blood pressure, cardiac output, stroke volume, total peripheral resistance, or ECGs were noted in any group.

28. Hemodynamic Effects of BM 06.022 in a Canine Stenotic Model of Coronary Thrombosis
Batch #7/88, 2/89, 5/89, 9/89
Anesthetized beagle dogs with an open-chest preparation of coronary thrombosis were single IV injected at 50, 100, 140, or 200 kU/kg BM 06.022 (90 mins) or 200, 800, 1130, or 1600 kU/kg alteplase. Mean arterial blood pressure declined in all BM 06.022 & alteplase groups, but heart rate remained constant. [Per the sponsor] the changes resulted from blood loss via the plug lysis of the surgical wounds, as this hemodynamic change was not noted in conscious dogs (study #27).

29. Hemodynamic Effects of High Doses of BM 06.022 in Conscious Dogs

Batch #821 403A (lyophilized); 578 kU/mg
Beagle dogs with chronically implanted catheters were single IV injected at 0.42 (3-fold effective dose in dogs) or 1.4 MU/kg BM 06.022 (2 mins). A dose of 1.4 MU/kg resulted in inconsistent hemodynamic changes in 2/4 dogs - blood pressure drop + bradycardia in one dog and increased blood pressure + tachycardia in the other dog. There were no significant changes in prekallikrein levels - thus there was no evidence for bradykinin-induced hypotension. [The sponsor suspected the catheter implants may have had fibrin clots, which were dispersed by BM 06.022 and cites such inconsistencies in alteplase studies.]

30. Antigenicity Study of BM 06.022 in Guinea Pigs

Batch #821 699A; 556 kU/mg
Active Systemic Anaphylaxis - Hartley ♂ guinea pigs (5/grp) were sensitized via IV injection of 0.25 or 1.25 mg/kg BM 06.022 or SC injection of 0.75 mg/kg BM 06.022 (+Freund's complete adjuvant); every other day for 3 injections total. On the 19th day after the 1st sensitization, the pigs were challenged (IV injection) with 1.25 mg/kg BM 06.022. A total of 5/5, 4/5, & 5/5 pigs dosed at 0.25 (IV), 1.25 (IV), or 0.75 (SC) mg/kg showed ASA symptoms.

Passive Cutaneous Anaphylaxis - Hartley ♂ guinea pigs (5/grp) were sensitized via ID injection of the sera from the ASA-sensitized pigs, followed (4 hours later) by IV injection of 0.5 or 1.25 mg/kg BM 06.022 + 1% Evans blue dye solution. The pigs were killed after 30 minutes, and the diameter of the blue dye spot leakage (on the dorsal skin surface) measured. At the 0.5 mg/kg challenge dose, 4/5, 1/5, & 5/5 pigs sensitized with 0.25 (IV), 1.25 (IV), or 0.75 (SC) mg/kg BM 06.022 had positive reactions. [At 1.25 mg/kg, systemic skin-bluing occurred - attributed (by the sponsor) to high arginine concentration required to dissolve BM 06.022.

EIA - The sera of Hartley ♂ guinea pigs (5/grp) from the ASA study were collected 16 days after the 1st sensitization. The IgG titres were positive.

31. Comparative Evaluation of the Antigenicity of BM 06.022 in Dogs

Batch #9/89; 515 kU/mg
Beagle dogs were IV injected at 140 kU/kg/day BM 06.022 for 14 days, followed by a single challenge injection on day 28. Additional dogs were IV infused at 800 kU/kg/day alteplase for 14

detection of antibodies specific for each material were present in week 2. Crossreactivity of antibodies were noted for both products and competitive binding of BM 06.022-antibodies to alteplase as the excess free antigen was shown [antigenic epitopes for both materials were the same]. In addition, both products caused slight hypotension and bradycardia during week 2 and upon rechallenge.

32. Fibrin-Selectivity of BM 06.022 in Rabbits After Single IV Bolus Injection
Batch #821 403A (lyophilized); 578 kU/mg
NZW rabbits were IV injected at 50, 100, 200, or 400 kU/kg of BM 06.022 (15 sec) or with alteplase at 400, 800, or 1600 kU/kg (15 sec). Dose-dependent plasmin generation (plasminogen depletion), consumption of α2-antiplasmin, and decrease of fibrinogen occurred for both products. The ED_{50} for 50% thrombolysis was 163 & 871 kU/kg for BM 06.022 and alteplase, respectively. Slightly prolonged PTTs were noted at 400 kU/kg BM 06.022 (compared to controls). Platelet counts were slightly reduced by both products (compared to controls).

33. Effects of the Novel Recombinant Plasminogen Activator BM 06.022 on Platelets and Bleeding Time in Rabbits; and by Martin, U., et al., Platelets. 3:247-253, 1992.
Batch #821 699A; 556 kU/mg
NZW rabbits that were pretreated with saline or acetylsalicylic acid (ASA) were IV irjected at 200 kU/kg BM 06.022 or with 800 kU/ kg alteplase. At 2 hours: Both thrombolytics prolonged bleeding times in rabbits pretreated with saline or ASA - from 2 to 3.9 min for BM 06.022 alone and from 2.8 to 3.8 min for alteplase alone. Bleeding times were further prolonged with the ASA combination. The prolongation was reversed by 1-desamino-8-D-arginine vasopressin (DDAVP), by aprotinin, and/or by tranexamic acid. Platelet counts were not reduced by BM 06.022 alone or with ASA. Platelet aggregation was not affected by BM 06.022 or alteplase alone. In ASA pretreated rabbits, platelet aggregation was reduced by BM 06.022, not by alteplase.

Comments:
- Tranexamic acid, a lysine-binding analog, inhibits plasminogen binding to platelets stimulated by thrombin.

DDAVP is known to reverse the increased vascular permeability caused by plasminogen activators.

Aprotinin inhibits plasmin.

• The sponsor comments that aggregation studies should be repeated in a more extensive manner, C

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34. Effects of BM 06.022 on Simplate Bleeding Time in Anesthetized Dogs

Batch #7/88, 2/89, 5/89, 9/89

Anesthetized, heparinized, open-chest beagle dogs were single IV injected at 50, 100, 140, or 200 kU/kg (bolus) or at 50 or 140 kU/kg (90 min) of BM 06.022. Additional dogs were single IV injected at 200, 800, 1130, or 1600 kU/kg (bolus) or 800 kU/kg (90 min) of alteplase. Injection or infusion of BM 06.022 did not (statistically) significantly prolong bleeding times (maximum increases of 13-44% over baseline). Injection or infusion of up to 800 kU/kg alteplase did not (statistically) significantly prolong bleeding times (maximum increases of 23-32% over baseline). Injection of 1130 or 1600 kU/kg alteplase prolonged bleeding time (from 2.4 min to 6.1 min), thus presents a risk of intracerebral bleeding complications.

35. Effects of BM 06.022 on Hemostasis Parameters After IV Administration to Anesthetized Dogs

Batch #7/88, 2/89, 5/89, 9/89

Anesthetized beagle dogs were single IV injected at 50, 100, 140, or 200 kU/kg (bolus) or at 140 kU/kg (90 min) of BM 06.022. Additional dogs were single IV infused at 200, 800, 1130, or 1600 kU/kg (bolus) or 800 kU/kg (90 min) of alteplase. Effects of BM 06.022 and alteplase were dose-dependent. At equi-effective treatment regimens of 140 kU/kg BM 06.022 injection vs. 800 kU/kg alteplase infusion, hemostasis data were as follows:

Compound	$AUC_{0-2\,h}$ (IU.hr/mL)	Fibrinogen (%)	Plasminogen (%)	α2-antiplasmin (%)	Platelets (%)
Alteplase	490 ±85	97 ±5	84 ±1	72 ±9	100 ±6
BM 06.022	554 ±62	93 ±1	81 ±1	48 ±8	104 ±5

The higher AUC for BM 06.022 resulted in a greater decrease in plasminogen and α2-antiplasmin.

36. Effects of BM 06.022 on the Hemostatic System After Single IV Bolus Injection into Non-Human Primates
Batch #2/89, 9/89
Non-human ♀ primates (Macaca arctoides) were single IV injected at 200 kU/kg (1 min bolus) of BM 06.022 or injected at 200 kU/kg (1 min bolus) of alteplase. Both BM 06.022 and alteplase did not cause any alterations in fibrinogen, plasminogen, α2-antiplasmin, platelets, or hematocrit at 0 and 2 hours post-injection.
Note that the dose of alteplase was known to induce only marginal thrombolytic effects in dogs.

Comment:
- [Per the sponsor] The BM 06.022 dose used had been shown to induce maximum thrombolytic effect in dogs, but a thrombolysis study in primates was not considered ethical.

PRECLINICAL TOXICOLOGY STUDIES:
List of Studies:

1. Acute Toxicity of BM 06.022 IV in Rats; study #F2; performed (per GLP) at BM

2. Acute Toxicity of BM 06.022 Rabbits, IV; study #F1; performed (per GLP) at BM

3. Single Dose IV Toxicity Study in the Cynomolgus Monkey with a 14-Day Observation Period; study #F3; performed (per GLP) at

4. BM 06.022 (rPA) 2-Week IV Toxicity Study in Rats; study #G1; performed (per GLP) at BM

5. 2-Week Toxicity Study of BM 06.022 Dog IV; study #G2; performed (per GLP) at BM

6. Repeated Dose Toxicity Study of BM 06.022 Dog IV; study #G2; performed (per GLP) at BM

7. Repeated Dose Toxicity Study of BM 06.022 Administered IV to Cynomolgus Monkeys for 2 Weeks; study #G3; performed (per GLP) at

8. Local IV Tolerance of BM 06.022 in the Rabbit; study #F101; performed (per GLP) at BM

9. Local IV Tolerance of BM 06.022 in Rabbits (Slow Bolus); study #F102; performed (per GLP) at BM

11. Local Paravenous Tolerance of BM 06.022 (rPA) in Rabbits; study #F104; performed (per GLP) at BM

12. Local IV Tolerance of Different Solutions of BM 06.022 (rPA)/Arginine Chloride Formulations in Rabbits; study #F105; performed (per GLP) at BM

Acute Toxicity Studies

1. Acute Toxicity of BM 06.022 IV in Rats
Batch #: 821 564A
Species: Crl:CD SD rats (5/sex/group)
Dose Level: 0, 4.2, 8.4 MU/kg
Route/Duration: IV/single dose + 14-day observation period
Findings: No deaths; slight apathy postdose (~30 minutes duration) in 8.4 MU/kg rats

2. Acute Toxicity of BM 06.022 Rabbits, IV
Batch #: 821 405A
Species: cross-bred rabbits (5/sex/group)
Dose Level: 0, 4.2 MU/kg [1 MU = 1.73 mg]
Route/Duration: IV/single dose + 14-day observation period
Findings: No deaths; slight apathy immediately postdose [presumed by the sponsor to be a drop in blood pressure, based on earlier canine studies].
When the rabbits were bled at 2 hours postdose, prolonged and more intense bleeding from the collection site occurred. Increased PTT and TT and ↓ fibrinogen levels occurred postdose; but were at baseline by 24 hours postdose. Beginning on day 2/3, hematuria was evident in all rabbits - from minimal to highly positive. By the end of the study minimal hematuria was sporadically noted.

3. Single Dose IV Toxicity Study in the Cynomolgus Monkey with a 14-Day Observation Period
Batch #: 821 699 80A
Species: cynomolgus monkeys (2/sex/group)
Dose Level: 4.2, 8.4 MU/kg [1 MU = 1.80 mg]
Route/Duration: IV/single dose + 17/18-day observation period
Findings: No deaths
Day 1 - postdose - [sedative effect] slight apathy/ unconsciousness - 2/4 (4.2 MU/kg), 3/4 (8.4 MU/kg), ending 15-90 minutes postdose

↓ blood pressure - immediately postdose

Epistaxis - 2/4 (8.4 MU/kg) - postdose

Injection site & blood sampling sites - peeling skin, progressing to sores, healing by days 7-13.

↓ appetite for both groups - week 1

Clinical Pathology - 3 hours postdose - not detectable PT, PTT, fibrinogen, except for 1/4 (4.2 MU/kg) monkeys had a PTT of 85.7 sec (compared to 17.1 sec baseline).

↓ plasmin, α2-antiplasmin - all animals

Day 2 - slight ↓ red cell mass - all animals
↑ blood urea - dose-related
↓ total protein
↑ AST
↑ HGB, RBCs, protein - urine

Values returned to baseline by day 14

Histo - Injection site - SC hemorrhage

Comment:
- The unconsciousness was most likely due to transient hypotension.

Multidose Toxicity Studies

4. BM 06.022 (rPA) 2-Week IV Toxicity Study in Rats
Batch #: 821 403A, 821 405A (pool #443 339)
Species: SPF Crl:CD(SD) BR rats (10/sex/group)
Dose Level: 0, 0.14, 0.42, 1.40 MU/kg/day
Route/Duration: IV/14 days + 12-day observation period
Methods: Clinical signs, BWs, food consumption, ophthalmology, clinical pathology (baseline, weeks 1 & 2, recovery), and gross and histopathology were performed.

Findings: No deaths; no abnormal clinical sign, BW, food consumption, ophthalmology, clinical pathology, gross pathology, organ weight, and histopathology data were noted.

The NOEL = 1.4 MU/kg/day.

Comment:
• [Per the sponsor] The 0.14 MU/kg/day dose corresponds to the human therapeutic dose; while 1.4 MU/kg/day corresponds to 10-fold the therapeutic dose.

5. 2-Week Toxicity Study of BM 06.022 Dog IV
Batch #: 821 403A, 821 404A, 821 405A (pool #443 339)
[1 MU = 1.73 mg]
Species: beagle dogs (3/sex/group)
Dose Level: 0, 0.14, 0.42, 1.40 MU/kg/day
Route/Duration: IV/14 days + 11-day observation period
Methods: Clinical signs, BWs, food consumption, ophthalmology, ECGs, clinical pathology (baseline, weeks 1 & 2, recovery), anti-BM 06.022 antibodies, and gross and histopathology were performed.

Findings: No deaths; no abnormal BW, food consumption, ECG, ophthalmology, clinical chemistry & urinalysis, gross pathology, organ weight, and histopathology data were noted.

Clinical Signs - Week 2 - 1/6 (0.14 MU/kg), 4/6 (0.42 MU/kg), & 4/6 (1.4 MU/kg) dogs displayed signs of collapse/shock (report wording) immediately after injection, progressing to prostration (lasting ~2 minutes). **Scheduled ECG data were normal.** ECGs were taken at baseline and on days 1, 4, 8, & 15, at 2-3 hours postdose.

Note that the 0.14 MU/kg dog was also positive for antibodies.

One 1.4 MU/kg ♀ showed allergy-like signs on day 4 - slight swelling of the palpebral conjunctivae and urticaria in the abdominal region.

Antibodies - day 14 - 0.14 MU/kg - one ♀ was markedly positive; two others weakly positive
0.42 & 1.4 MU/kg - all animals were positive

Comment:
• The sponsor cites two potential reasons for the collapse of the dogs postdose:
1. The clinical signs are attributed to a probable sudden drop in blood pressure - but no data are provided to support this. The sponsor cites study #E7 - "Hemodynamic Effects of High Doses of BM 06.022 in Conscious Dogs", in which a dose of 1.4 MU/kg resulted in inconsistent hemodynamic changes in 2/4 dogs - blood pressure drop + bradycardia in one dog and increased blood pressure + tachycardia in the other dog.

2. A relationship may exist between the production of anti-BM 06.022 antibodies and the adverse effects that occurred after dosing because the signs were not present until day 9 and were noted only in those animals with antibody development.

Clinical Hematology - No difference in TT or PTT.
Day 14 - predose #14 - ↓ fibrinogen, plasminogen, α2-antiplasmin [compared to control & baseline] - all treated groups - dose-dependent

Day 14 - postdose #14 (2 hrs) - the values were even further ↓ - dose-dependent

The NOEL was not achieved.

7. Repeated Dose Toxicity Study of BM 06.022 Administered IV to Cynomolgus Monkeys for 2 Weeks
Batch #: 780 141 80
Species: cynomolgus monkeys (2/sex/group)
Dose Level: 0, 0.34, 1.04, 3.11 MU/kg/day [0, 0.6, 1.8, 5.4 mg/kg]
Route/Duration: IV (antebrachial vein [elbow]) /14 days
Methods: Clinical signs, BWs, appetite, ophthalmology, ECGs, clinical pathology (baseline & day 13), anti-BM 06.022 antibodies, and gross and histopathology were performed.

Findings: No deaths
Clinical Signs -
Pale mucosa - 3.11 MU/kg ♀s - days 6-14

Reddening of skin - 3/4 animals (0.34 MU/kg); 2/4 (1.04 MU/kg); 1/4 (3.11 MU/kg) - days ~7-14

Injection site swelling, reddening - 2/4 animals (0.34 MU/kg); 4/4 (1.04 MU/kg); 4/4 (3.11 MU/kg) - days ~2-14 + 1/4 control (days 13, 14)

Elastic difficulty of the elbow - 1/4 each at 0.34 & 3.11 MU/kg - days 12-14

↓ blood pressure - 1/4 (1.04 MU/kg); 2/4 (3.11 MU/kg) - one hour postdose

Antibodies - 10/12 treated animals were positive at week 2

Clinical Pathology -
↓ red cell mass; ↑ retics, MCV, erythroblasts - all treated

Gross - Injection site - hemorrhage/hematoma - 1/4 (control); 2/4 (0.34 MU/kg); 3/4 (1.04 MU/kg); 4/4 (3.11 MU/kg)

Right adrenal - enlargement/hemorrhage - 1/4 (3.11 MU/kg - also exhibited hypotension)

Histopathology - Injection site - hemorrhage, edema, infiltration of neutrophils & other leukocytes [higher incidence in treated groups]

Elbow - synovitis - 1/4 each at 0.34 & 3.11 MU/kg **[probably due to the injection technique to the cubitus]**

Spleen lymph follicle - extramedullary hematopoiesis/atrophy - 1/4 each at 0.34 & 1.04 MU/kg

Adrenal - hemorrhage & parenchymal necrosis - 1/4 (3.11 MU/kg)

The NOAEL was 1.04 MU/kg/day.

8. Local IV Tolerance of BM 06.022 in the Rabbit
Batch #: 821 403A
Species: cross-bred rabbits (5/group)
Dose Level: 0, 5 MU in 4.6 mL distilled water; 5 MU in 7.1 mL distilled water; 1 mL injected (injection rate not given)
Route/Duration: IV/single dose
Findings: [killed 24 hrs postdose] - 5 MU in 4.6 mL = 2/5 rabbits - paravenous tissue irritation; 2/5 rabbits - loss of endothelium; 5/5 rabbits - focal paravenous bleeding + edema
Placebo = 1/5 rabbits - loss of endothelium; 3/5 rabbits - focal paravenous bleeding + edema

5 MU in 7.1 mL = 1/5 rabbits - loss of endothelium; 1/5 rabbits - paravenous tissue irritation; 4/5 rabbits - focal paravenous bleeding + edema
Placebo = 1/5 rabbits - loss of endothelium; 2/5 rabbits - focal paravenous bleeding + edema

Comment:
- To some extent, the findings can be linked to the injection technique, as placebo animals also displayed lesions.

9. Local IV Tolerance of BM 06.022 in Rabbits (Slow Bolus)
Batch #: 821 564A
Species: cross-bred rabbits (5/group)
Dose Level: 0, 1 MU/mL; 1 mL injected
Route/Duration: IV/single dose (2 min injection)
Findings: [animals killed 24 hrs postdose] - focal paravenous bleeding &/or edema were noted in comparable numbers in both placebo & treated rabbits.

10. Acute Local Intra-arterial Tolerance of BM 06.022 (rPA)
Batch #: 821 699A
Species: cross-bred rabbits (5/group)
Dose Level: 0, 1 MU/mL; 0.5 mL injected (injection rate not given)
Route/Duration: IV/single dose
Findings: [animals killed 7 days postdose] - focal paravenous bleeding &/or edema were noted in comparable numbers in both placebo & treated rabbits.

11. Local Paravenous Tolerance of BM 06.022 (rPA) in Rabbits
Batch #: 780 244 80A
Species: cross-bred rabbits (5/group)
Dose Level: 0, 1 MU/mL; 0.5 mL injected (injection rate not given)
Route/Duration: paravenous (injection into area of marginal ear vein)/single dose
Findings: [animals killed 72 hrs postdose] - ears bluish-red discoloration, with swelling/warmness - 5/5 rabbits

Severe focal hemorrhage; severe inflammatory edema with eosinophilic fluid; ulceration of epidermal layer with scabs; severe venous congestion of surrounding tissue - 5/5 rabbits

Control - 1/5 rabbits - slight focal hemorrhage; 3/5 rabbits - slight focal edema

Comment:
- [Per the sponsor] The findings were enhanced by BM 06.022.

12. Local IV Tolerance of Different Solutions of BM 06.022 (rPA)/Arginine Chloride Formulations in Rabbits
Batch #: KN446670; 91/0590; 91/0591 - **this formulation is not used clinically**
Species: cross-bred rabbits (5/group)
Dose Level: 0, 0.64 MU/ml (0.32 M arginine chloride), 0.8 MU/mL

Route/Duration: IV/single dose
Findings: [animals killed 24 hrs postdose] - ears with slight focal swelling &/or bluish-red discoloration - seen among all groups

Damage to venous wall at injection site - loss of endothelium, focal necrosis of the media, damage of the adventitia
Perivascular irritation - hemorrhage, edema, cellular infiltration
Acute intravascular thrombi - injection site
Incidence & severity of findings decreased at lower arginine chloride concentrations and lesions associated with veins injected with placebo + arginine chloride were notably less severe compared to BM 06.022.

Comment:
● [Per the sponsor] The findings correspond to prolongation of bleeding (not evaluated) - which should not be a concern in humans, as the product is administered via a deep IV injection.

Reproduction/Teratology Studies

List of Studies:

1. Study on Administration of BM 06.022 Prior to and in the Early Stages of Pregnancy in Rats; study #K1; performed (per GLP) at

2. Study on Administration of BM 06.022 During the Period of Organogenesis in Rats; study #K2; performed (per GLP) at

3. A Preliminary Study on the IV Administration of BM 06.022 During the Period of Organogenesis in Rabbits (seq II); study #K3; performed (per GLP) at

1. Study on Administration of BM 06.022 Prior to and in the Early Stages of Pregnancy in Rats
Batch #: 780 141 80 Ac
Species: Crj:CD SD rats (24/sex/group)
Dose Level: 0, 0.75, 2.5, 7.5 mg/kg/day
Route: IV
♂s - dosed 63 days prior to mating & during mating; killed after confirmation of pregnancy
♀s - 14 days prior to mating, during mating, & until GD 7; killed

Findings: ♀s - depression of spontaneous movement - ≥2.5 mg/kg; no effect on resorptions, implantations, or corpora lutea

♂s - depression of spontaneous movement - ≥2.5 mg/kg; paleness; ↓ BW gain - 7.5 mg/kg; dose-dependent ↑ spleen & heart weight; ↑ extramedullary hematopoiesis

♂s & ♀s - glomerulonephritis; hepatocyte swelling & necrosis; ectopic calcification of the heart & other organs; and edema in various organs - all groups

F_1 fetuses - no effects on survival, weight, or external findings

The NOEL was <0.75 mg/kg/day (0.43 MU/kg) for the F_0 generation and 7.5 mg/kg/day (4.31 MU/kg) for reproductive effects and fetal toxicity.

Comments:
- The paleness, ↑ splenic weight and extramedullary hematopoiesis were probably due to anemia associated with the pharmacological action of chronic exposure to BM 06.022.

- The microscopic findings noted in the F_0 rats - including controls - seems to be rather severe for such a short study duration.

- The dose in MU/kg was not given in the report. The sponsor provided the correlation in MU/kg: 0.43, 1.44, 4.31 MU/kg for 0.75, 2.5, & 7.5 mg/kg, respectively.

2. Study on Administration of BM 06.022 During the Period of Organogenesis in Rats
Batch #: 780 141 80 Ac
Species: Crj:CD SD rats (36 ♀s/group)
Dose Level: 0, 0.75, 2.5, 7.5 mg/kg/day
Route/Duration: IV/GD 7-17
1. ~23 ♀/dose killed on GD 20; ~13 ♀/dose littered
2. F_1 fetuses - 1/3 examined for visceral changes; 2/3 examined for skeletal changes
3. F_1 pups evaluated for developmental changes (i.e., pupillary reflex, pinna detachment, incisor eruption, etc...)
4. Selected F_1 pups killed on LD 21
5. Remaining F_1 pups evaluated for gonadal development & behavioral tests (i.e., open field, water maze)
6. Remaining F_1 pups mated; dams killed on GD 20
7. F_2 fetuses examined for external/visceral/skeletal changes

Findings: F_0 - depression of spontaneous movement - ≥2.5 mg/kg; no maternal or embryo/fetal toxicity
F_1 - no effects on development/behavior, mating, and all other physical parameters
F_2 - no effects

The NOEL was <0.75 mg/kg/day for the F_0 generation and 7.5 mg/kg/day for reproductive effects and F_1/F_2 toxicity.

3. A Preliminary Study on the IV Administration of BM 06.022 During the Period of Organogenesis in Rabbits (seg II)
Batch #: 780 141 80 Ac
Species: SPF Japanese white rabbits
Dose Level: 0, 0.5, 1.5 mg/kg/day- 3 ♀s/grp
5.0 mg/kg/day - 2 ♀s
7.5 mg/kg/day - 1 ♀
Route/Duration: IV/GD 6-18; killed on GD 29

Findings: F_0 - vaginal bleeding & discoloration of eyeballs - beginning GD 14, followed by abortion - 2/3 (1.5 mg/kg - on GD 17 & 18) and 1/2 (5 mg/kg - on GD 16)

Not pregnant - 1/2 (5 mg/kg), 1/1 (7.5 mg/kg)

Vaginal bleeding - 1/3 (0.5 mg/kg - GD 19)
Prolonged hemostasis - GD 7-11 - ≥0.5 mg/kg
↓ BW, food consumption - 1.5, 5 mg/kg
Uterine retention of blood - 1/3 (0.5, 1.5 mg/kg)

No embryo/fetal toxicity was noted.

Comment:
- The dose in MU/kg was not given in the report. The sponsor provided the correlation in MU/kg: 0.29, 0.86, 2.88, 4.31 MU/kg for 0.5, 1.5, 5.0, & 7.5 mg/kg, respectively.

Mutagenicity Studies

List of Studies:

1. Mutagenicity of BM 06.022 (rPA). Standard Ames Plate Incorporation Assay; study #J2; performed (per GLP) at BM

2. Mutagenicity of BM 06.022 (rPA). Standard Ames Plate Incorporation Assay with S. typhimurium and E. coli; study #J5; performed (per GLP) at BM

3. Mutagenicity of BM 06.022 (rPA). Chromosome Aberration Test with Chinese Hamster V79 Cells in vitro; study #J6; performed (per GLP) at BM

4. Mutagenicity of BM 06.022 (rPA). Chromosome Aberration Test with Human Lymphocytes Cultured in vitro; study #J7; performed (per GLP) at BM

5. Gene Mutation of Chinese Hamster V79 Cells in vitro (V79/HGPRT) with BM 06.022 (rPA); study #J8; performed (per GLP) at

6. Gene Mutation of Chinese Hamster V79 Cells; study #J9; performed (per GLP) at

7. Mutagenicity of BM 06.022 (rPA). Dose Range Finding Study for the in vivo Micronucleus Test with NMRI Mice; study #J3; performed (per GLP) at BM

8. Mutagenicity of BM 06.022 (rPA). IV in vivo Micronucleus Test with Bone Marrow Erythrocytes in the Mouse; study #J4; performed (per GLP) at BM

9. Mutagenicity of BM 06.022. In vivo Long Term Micronucleus Test with Bone Marrow Erythrocytes of SD Rats; study #J1; performed (per GLP) at BM

In Vitro Non-Mammalian System

1. Mutagenicity of BM 06.022 (rPA). Standard Ames Plate Incorporation Assay
Batch #: 171089 (24.7 mg/mL); 220889 (27.0 mg/mL)
Strains: S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538 ± S9
Dose Level: 0, 10.4, 34.7, 104, 347, 1040 KU/plate
Findings: No statistically significant increase of revertant numbers were noted

2. Mutagenicity of BM 06.022 (rPA). Standard Ames Plate Incorporation Assay with S. typhimurium and E. coli
Batch #: 780 244 80 A
Strains: S. typhimurium TA 98, TA 100, TA 1535, TA 1537 ± S9
E. coli WP2uvr^{A} ± S9
Dose Level: 0, 2, 6, 20, 60, 200 KU/plate
Findings: No statistically significant increase of revertant numbers were noted

In Vitro Mammalian System

3. Mutagenicity of BM 06.022 (rPA). Chromosome Aberration Test with Chinese Hamster V79 Cells in vitro
Batch #: 780 320 80 A
Cell: Chinese hamster V79 cells ± S9
Dose Level: 0, 0.2, 2.0, 20 KU/plate
Findings: No evidence of in vitro clastogenic activity was found

4. Mutagenicity of BM 06.022 (rPA). Chromosome Aberration Test with Human Lymphocytes Cultured in vitro
Batch #: 780 141 80 Ac
Cell: Human peripheral lymphocytes ± S9
Dose Level: 0, 2, 6, 20 KU/plate
Findings: No evidence of clastogenic activity was found

5. Gene Mutation of Chinese Hamster V79 Cells in vitro (V79/HGPRT) with BM 06.022 (rPA)
Batch #: 780 320 80A; 780 244 80A; 780 514 80A
Cell: Chinese hamster V79 cells ± S9
Dose Level: 0, 10, 30, 40, 50, 70, 100 KU/mL
Findings: No evidence of clastogenic activity was found
[Note this study was performed in 1992.]

6. Gene Mutation of Chinese Hamster V79 Cells
Batch #: 781 111 80A
Cell: Chinese hamster V79 cells ± S9
Dose Level: 0, 10, 30, 40, 50, 70, 100 KU/mL
Findings: No evidence of clastogenic activity was found
[Note this study was performed in 1995.]

In Vivo Mammalian System

7. Mutagenicity of BM 06.022 (rPA). Dose Range Finding Study for the in vivo Micronucleus Test with NMRI Mice
Batch #: 780 141 80 Ac
Species:NMRI mice (2/sex/grp)
Dose Level: 0, 0.5, 1, 2, 4, 6, 8 MU/kg
Route: IV
Findings: No evidence of systemic toxicity after 72-hour observation period [results to be used for definitive study]

8. Mutagenicity of BM 06.022 (rPA). IV in vivo Micronucleus Test with Bone Marrow Erythrocytes in the Mouse
Batch #: 780 244 80 A
Species:NMRI mice (5/sex/grp)
Dose Level: 0, 2, 4, 8 MU/kg
Route: IV
Findings: No evidence of systemic toxicity after 12, 24, 48-hour observation/sampling periods
The polychromatic/normochromatic (PCE/NCE) ratio was not affected.
No indication of chromosome or spindle-function damage [as exhibited by the induction of micronuclei] was noted.

9. Mutagenicity of BM 06.022. In vivo Long Term Micronucleus Test with Bone Marrow Erythrocytes of SD Rats
Batch #: 443 339
Species:NMRI mice (5/sex/grp)
Dose Level: 0, 0.14, 0.42, 1.4 MU/kg
Route: IV
Findings: No evidence of systemic toxicity after 24-hour observation/sampling periods
The polychromatic/normochromatic (PCE/NCE) ratio was not affected.
No indication of chromosome or spindle-function damage [as exhibited by the induction of micronuclei] was noted.

CONCLUSION:

The recommended dosage for reteplase is as a 10+10 U double-bolus IV injection (not to exceed 2 minutes per injection). The second bolus is given 30 minutes after the first injection.

The preclinical studies presented from PLA #95-1167 depicted a higher thrombolytic potency compared to other thrombolytics, such as alteplase, primarily due to the kinetic profile. In a rabbit model of jugular vein thrombosis, the ED_{50} was 94 kU/kg for reteplase and 436 kU/kg for alteplase. Similar findings were shown in a canine stenotic model of coronary artery thrombosis. In addition, in the canine model, it was shown that an IV bolus injection of 140 kU/kg reteplase was equally effective as a 90-minute infusion of 800 kU/kg alteplase.

Thrombocytopenia did not occur after injection of pharmacologic levels of reteplase in studies performed in rabbits and dogs. There were no adverse effects on platelet aggregation or bleeding times. Concomitant administration of heparin did not prolong

Many species (rats, dogs, monkeys, rabbits, mice) were used in the performance of the toxicology studies - reteplase was pharmacologically active in all species used. The single dose IV bolus injection studies resulted in exaggerated pharmacologic effects including transient hypotension (apathy, unconsciousness) and hypoventilation in primates. Doses up to 8.4 MU/kg were tolerated. A 14-day rat IV study resulted in a NOEL of 1.4 MU/kg/day (maximum dose injected). Dogs IV injected at levels up to 1.4 MU/kg/day (for 14 days) displayed an allergic/anaphylactoid reaction during the second week of dosing, which appeared to parallel the development of anti-reteplase antibodies. No microscopic lesions were noted. Cynomolgus monkeys displayed expected pharmacodynamic effects at reteplase levels up to 3.11 MU/kg/day (for 14 days), including decreased red cell mass, injection site reddening/swelling, and transient hypotension. The NOAEL was 1.04 MU/kg/day, as one 3.11 MU/kg monkey also displayed unilateral hemorrhage and parenchymal necrosis of the adrenal gland. Based on body weight, the tolerated doses in the animal studies ranged from approximately 29-fold [in the single dose studies] to 3-fold [in the repeated dose studies] higher relative to the human dose.

Segment I and II reproductive toxicity studies in rats revealed a NOEL of <0.43 MU/kg/day for the F_0 generation (1.5-fold times the human dose) and 4.31 MU/kg/day for reproductive effects and fetal toxicity (15-fold times the human dose). The females and males displayed hypoactivity (likely related to hypotension). An abortifacient effect in mid-gestation, as well as genital tract hemorrhaging, was noted in a segment II study in rabbits at doses of 0.86 MU/kg/day (3-fold higher than the human dose).

No mutagenic potential was exhibited via in vitro non-mammalian systems, in vitro mammalian systems, or via in vivo mammalian systems.

The preclinical data adequately support use of the product, reteplase or Rapilysin™, for the dosage regimen specified by the sponsor.

There are no requirements or requests to be made of the sponsor at this time.

Mercedes A. Serabian, M.S., D.A.B.T., Toxicologist 2/27/96

Key Words: recombinant human plasminogen activator; reteplase; Rapilysin™; rPA; AMI; thrombi; congestive heart failure; tPA; streptokinase; urokinase

Concurrences:
OTRR/C,P-T/MGreen

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